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Disposition of diazepam in young and elderly subjects after acute and chronic dosing

ROBERT J. HERMAN & GRANT R. WILKINSON
Departments of Medicine and Pharmacology, Vanderbilt University, Nashville, Tennessee, USA and the University of Saskatchewan, Saskatoon, Saskatchewan, Canada

1 The pharmacokinetics of diazepam were examined in seven young (20–30 years) and six elderly (60–75 years) males prior to and also after chronic oral dosing of diazepam.

2 Following intravenous administration, the half-life and volume of distribution of ^{14}C-labelled diazepam in the elderly were approximately twofold greater than corresponding estimates in younger subjects (mean $\pm$s.d., 71.5 ± 27.6 *vs* 44.5 ± 16.5 h and 1.39 ± 0.32 *vs* 0.88 ± 0.30 l kg^{-1}, respectively). Clearance did not differ between the two groups (0.26 ± 0.09 *vs* 0.29 ± 0.09 ml min^{-1} kg^{-1}).

3 The accumulation of diazepam and its major metabolite, desmethyldiazepam, were extensive during chronic administration. A radioreceptor assay that measured total benzodiazepine activity, including diazepam and its active metabolites, indicated that the accumulation of 'benzodiazepine equivalents' was similar to the sum of the accumulated diazepam and desmethyldiazepam concentration levels. However, the level of 'benzodiazepine equivalents' on multiple-dosing was about double that of the predicted steady-state 'equivalent' concentration from single-dose studies. This was due to the insensitivity of the radioreceptor assay for desmethyldiazepam following single-dose diazepam administration.

4 There were no age- or dosing-related differences in diazepam clearance (0.37 ± 0.22 *vs* 0.32 ± 0.18 ml min^{-1} kg^{-1}, young *vs* elderly, single-dose; 0.37 ± 0.11 *vs* 0.27 ± 0.12 ml min^{-1} kg^{-1}, young *vs* elderly, multiple-dose) and no age-related differences in the levels of accumulated 'benzodiazepine equivalents' (243.7 ± 60.1 *vs* 288.0 ± 125.8 ng ml^{-1}, young *vs* elderly).

5 Thus, changes that occur in diazepam disposition with ageing after acute administration do not appear to be important during chronic dosing. On the other hand, accumulation of diazepam and desmethyldiazepam are considerable and would be expected to be clinically relevant.

Keywords diazepam pharmacokinetics ageing chronic dosing radioreceptor assay

Introduction

The elderly often exhibit an increased sensitivity to the sedative effects of benzodiazepines [1–4]. While age-related changes in pharmacokinetics and/or pharmacodynamics have been proposed for many drugs in this class, several important questions remain. In the case of diazepam, increases in the elimination half-life with ageing appear to be due to alterations in its volume of distribution [5–8]. Reduced clearance, reflective of decreased drug metabolizing ability in the elderly, may also be contributory [6–8], although this difference has not been consistently observed [5, 9, 10]. Unfortunately, such findings have been limited to single-dose studies, whereas multiple dosing is frequently more clinically relevant. This consideration is particularly important for diazepam because its oxidative metabolites are eliminated more slowly than the parent drug and

Correspondence: Dr R. J. Herman, Department of Pharmacology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0.

accumulation occurs on multiple dosing. Moreover, all of these metabolites (desmethyldiazepam, oxazepam and temazepam) are pharmacologically active and, thus, contribute to the overall effects of the drug. Furthermore, it has been suggested that diazepam's elimination is impaired following repeated dosing due to inhibition of its metabolism by the drug, itself, or one or several of its metabolites [11, 12]. For these reasons, we decided to re-examine the age-related changes in diazepam disposition following both single-dose and multiple-dose administration. We also incorporated a non-specific radioreceptor assay capable of measuring 'total benzodiazepine activity' to be used along with conventional h.p.l.c.-based chemical determinations in order to evaluate the importance of the active metabolites.

Methods

Subjects and protocol

Thirteen healthy, non-smoking, males participated in the study. Seven were between the ages of 20 and 30 years (mean age, 25.3 ± 3.9 years; weight range, 57.6–90.0 kg) and six were 60–75 years (mean age, 68.3 ± 5.3 years; weight range, 69.2–98.4 kg). Sample size was calculated on the basis of the previously reported differences in diazepam kinetics [7, 10, 11] for an n sufficient to provide a power of 0.80 at $\alpha = 0.05$, as well as those differences which were considered to be clinically relevant (30–50%). All subjects were free of significant medical illness as evaluated by a medical history, physical examination and routine haematological and biochemical measurements of renal, hepatic and general metabolic function. We did not determine the polymorphic CYP 2C19 (mephenytoin) status of our volunteers. However, comparison of their subsequently measured diazepam clearances with reported values in poor and extensive metabolizer phenotypes [13] indicates that all individuals were extensive metabolizers at the CYP 2C19 locus. Subjects were asked to abstain from alcohol and/or other drugs beginning 2 weeks before and extending throughout the study period. The study was approved by the Vanderbilt University Institutional Review Board and written consent was obtained.

On the first study day, subjects presented themselves after an overnight fast. An intravenous cannula was established in the non-dominant arm for repeated blood sampling and maintained patent using a slow infusion of 0.9% saline. Following collection of blank samples, subjects were given an intravenous bolus injection of approximately 50 μCi (250 μg) of [2-^{14}C]-diazepam (specific activity 57 mCi mmol^{-1}, Amersham Corp., Arlington Heights, IL, USA) through the opposite arm together with 2 mg of non-labelled diazepam by the oral route. Blood was collected at frequent intervals over the ensuing 24 h and then daily, thereafter. On the seventh study day, subjects were started on a chronic dosing regimen consisting of diazepam 2 mg orally every 12 h with venous blood samples obtained just prior to the daily morning dose. At the beginning of the fifth week, and while oral diazepam administration continued, the simultaneous intravenous/oral dosing protocol and blood sampling were repeated. Seven days later diazepam was stopped, however, blood samples were collected daily for a further 1–2 weeks as drug and metabolites were eliminated.

Assay methods

Concentrations of diazepam, desmethyldiazepam, oxazepam and temazepam were measured in plasma by solvent extraction and h.p.l.c. Briefly, 1 ml of plasma was alkalinized with an equal volume of saturated sodium borate solution and 1.8×10^{-3} μCi of methyl-[^{3}H]-flunitrazepam (specific activity 60 Ci mmol^{-1}, DuPont-NEN, Boston, MA, USA) was added as an internal standard. Samples were extracted three times using freshly distilled diethyl ether and evaporated to dryness at 40° C under a nitrogen stream. The residue was then reconstituted in 100 μl of mobile phase and injected onto the chromatographic system. The latter consisted of a M6000A solvent delivery system, a 440 u.v.-detector and a U6K injector (Waters Associates, Milford, MA, USA). The mobile phase was a 1:1 mixture of methanol and distilled water delivered at a flow of 1.8 ml min^{-1}. Separation was obtained using a 3.9 mm × 25 cm, μBondapak C18 column (Waters Associates). Radiolabelled diazepam and metabolites were measured in the same samples by counting the radioactivity present in fractions collected during elution of the corresponding non-labelled peaks. Correction for extraction efficiency was based on the recovery of [^{3}H]-flunitrazepam in individual samples. Standard curves for diazepam, desmethyldiazepam, oxazepam and temazepam were linear over the concentration range of 5–1000 ng ml^{-1}, with a limit of measurement for each of approximately 3 ng ml^{-1} and intra- and inter-day assay coefficients of variability of 5% and 7%, respectively. Corresponding levels of radiolabelled drug and metabolites could be measured at least an order of magnitude lower with a limit of detection of about 0.1 ng ml^{-1}. Concentrations of non-labelled diazepam and desmethyldiazepam were estimated from h.p.l.c.-measured total concentrations (labelled plus non-labelled) less the contribution of the radiolabelled component.

Total plasma concentrations of biologically active benzodiazepines, herein referred to as 'benzodiazepine equivalents', were estimated using a radioreceptor assay [14, 15]. Specifically, this measured the competitive displacement of [^{3}H]-diazepam binding to rat cortical synaptosomes by non-labelled diazepam and metabolites in patient samples. Homogenates of rat cerebral cortex were prepared in 50 mmol Tris-HCl buffer (pH 7.4) and centrifuged for 5 min at 2000 ***g*** and 5° C. The supernatant was then centrifuged a further 10 min at 48,000 ***g*** to produce a crude P2-synaptosomal fraction [16]. This was re-suspended in Tris-HC1, whereupon duplicate aliquots were incubated with 0.54 nmol methyl-[^{3}H]-diazepam (specific activity 94 Ci mmol^{-1}, Amersham

Corp.) for 20 min at 0° C in the presence and absence of saturating concentrations (3.5 mmol) of non-labelled diazepam. The reaction was terminated by vacuum filtration over a Whatman GF/B filter which, after washing three times with ice cold Tris-HCl, was transferred to scintillation counting vials for measurement of radioactivity. [^{3}H]-diazepam binding, using this procedure, was saturable and specific with a K_d of 3.1–3.5 nmol, a B_{max} of 1.23×10^{-12} mol mg^{-1} of protein and a time to equilibrium of approximately 15 min. Competition curves for non-labelled diazepam and metabolites yielded K_i values of 3.7–8.6 nmol for diazepam, 6.1–6.6 nmol for desmethyldiazepam, 40.1 nmol for temazepam and 24.7 nmol for oxazepam. All of these are within reported ranges for binding to the putative benzodiazepine receptor [15–17].

Patient samples (150 µl plasma) containing unknown concentrations of 'benzodiazepine equivalents' were diluted with an equal volume of distilled water and completely deproteinized using 30 µl of 2 M perchloric acid. Duplicate 110 µl aliquots of the resulting supernatant were transferred to clean polypropylene tubes and these neutralized by the addition of 15 µl of 1 M KOH. Rat cortical synaptosomes (250 µl) and radiolabelled diazepam (25 µl) were then added and the binding of [^{3}H]-diazepam measured in the manner described. The concentrations of bindable benzodiazepines displacing [^{3}H]-diazepam in unknown samples were estimated through logit transformation and interpolation from competition curves obtained using known concentrations of diazepam and desmethyldiazepam. The limit of detection for the radioreceptor assay was 5 ng ml^{-1}, with an intra-assay coefficient of variation of 9% and an inter-assay coefficient of variation of 15%. Performance was monitored through measurement of quality controls and assays repeated whenever values fell outside their expected cumulative 95% confidence intervals.

Plasma protein binding was measured by equilibrium dialysis [18]. Trace quantities of [2-^{14}C]-diazepam were added to pre-study blank plasma and this dialyzed against 67 mmol phosphate buffer (pH 7.4) for 4 h using a Spectrapor® dialysis membrane (12,000–14,000 MW cut off). Radioactive samples were counted for 10 min with an ISOCAP 3000 scintillation counter (Tm-Analytic®, Elk Grove, IL, USA) using the external standard method to correct for counting efficiency.

Pharmacokinetic and statistical analyses

The plasma concentration-time profiles of intravenous diazepam were analysed by non-linear least squares regression and fitting with bi- and tri-exponential equations using the SAAM 23 program (Resource Faculty for Kinetic Analysis, Seattle, WA, USA) on a DEC-20 digital computer (Digital Equipment Corp., Maynard, MA, USA). The *F*-test [19] and Akaike Information Criterion [20] were used to determine the best statistical fit. Non-compartmental estimates of clearance (Dose/AUC), steady-state volume of distribution (Dose × AUMC/AUC^2) and half-life were obtained using conventional approaches [21], where AUC and AUMC refer to the areas under the zero and first-moment plasma concentration-time curves, respectively. Statistical analysis was by repeated measures ANOVA using the BMDP statistical package (University of California Press, Los Angeles, CA, USA) with $P \leq 0.05$ as the minimal level for acceptance of significance.

Results

Single-dose diazepam kinetics

Plasma concentrations of tracer doses of radiolabelled diazepam declined multiphasically after intravenous administration, and were best fitted by a tri-exponential equation in all study subjects, regardless of age. During the first few hours of measurement, levels declined more rapidly in the elderly than in the younger age group. However, subsequent elimination of the drug appeared to be somewhat slower (Figure 1a). This was confirmed by pharmacokinetic analysis (Table 1) which showed that the terminal elimination half-life of diazepam was approximately twofold greater in elderly subjects (mean difference 27 h, 95% CI 0–54). There was no statistically significant difference in the systemic clearance between the two groups (mean difference 0.03 ml min^{-1} kg^{-1}, 95% CI −0.08–0.14), although there was a trend towards a slightly reduced (15–25%) value in the elderly subgroup. The steady-state volume of distribution of diazepam, on the other hand, was markedly greater in older subjects (mean difference 0.51 l kg^{-1}, 95% CI 0.13–0.89), indicating that increased distribution was largely responsible for the prolongation in half-life. By contrast, the initial distribution volume showed no such age-related differences. Plasma protein binding of diazepam was extensive. However, the unbound fraction was similar in both young and elderly subjects, and pharmacokinetic parameters expressed in respect of unbound diazepam showed the same age-related effects as those estimated on the basis of total drug.

Absorption of diazepam following oral administration was rapid and complete and the elimination half-life as determined for this dose and route did not differ from that of the concomitantly administered radiolabelled intravenous drug (Figure 1b). Diazepam's apparent oral clearance was 5–10% greater than its systemic clearance consistent with a small first-pass effect and a high oral availability (Table 1). However, no age-associated differences were observed.

Multiple-dose diazepam kinetics

Comparison of diazepam clearances and half-lives following single-dose (oral and intravenous) administration with those obtained following multiple-dose oral administration showed no statistically significant differences. Accumulation of the end-of-dosage-interval

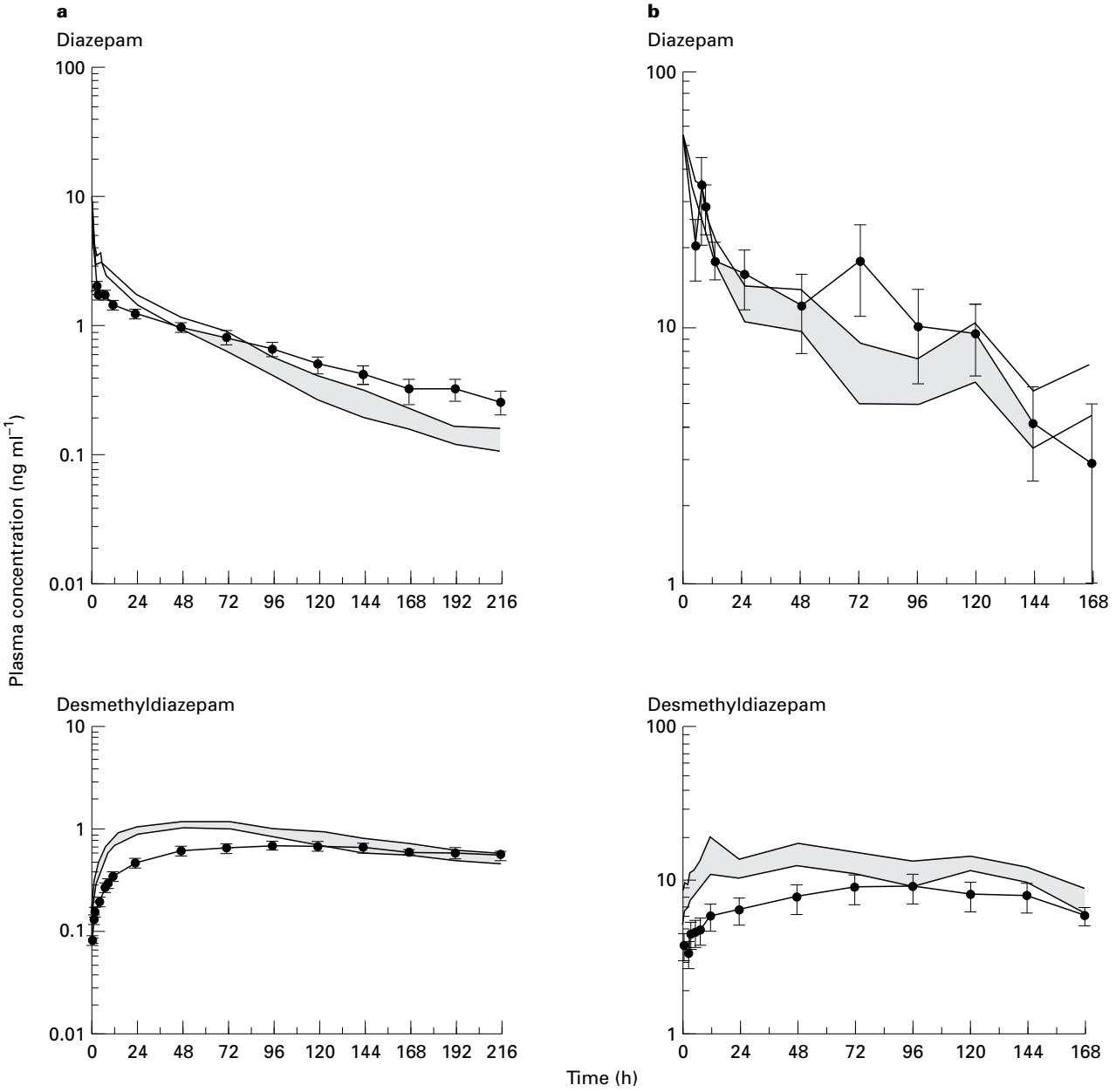

Figure 1 Plasma concentration-time profiles of diazepam and its metabolite, desmethyldiazepam, after single-dose intravenous (a) and oral (b) administration. The shaded area represents the ± 1 s.e. mean range on either side of the mean for the young group of subjects, whereas the mean results for the elderly group is indicated by separate data points with ± 1 s.e. mean bars. Note that concentrations of diazepam and desmethyldiazepam following the oral dose are about ten fold greater than after intravenous administration.

diazepam plasma concentrations occurred during chronic administration, with levels approaching plateau concentrations in 5 to 15 days in the young *vs* 8 to 24 days in the elderly (Figure 2a and b). Steady-state diazepam concentrations were higher in the elderly subgroup (147.9 ± 71.1 *vs* 90.9 ± 27.4 ng ml^{-1}), although variances were large and differences were only of borderline significance ($P = 0.06$, mean difference 57.0 ng ml^{-1}, 95% CI -10.0–100.0).

High plasma concentrations of desmethyldiazepam were found after both single and multiple dosing with the drug. However, only low levels of the 3-hydroxy metabolites, namely oxazepam and temazepam, were observed. Desmethyldiazepam elimination was extremely slow with a terminal half-life almost double that of diazepam (Figure 1(a)). During chronic dosing, desmethyldiazepam also accumulated—initial metabolite concentrations were lower than the parent drug, but by the time steady-state was achieved they either matched or exceeded those of diazepam. The extent of accumulation of desmethyldiazepam was greater in the elderly than in the young with steady-state plasma concentrations approaching 183.2 ± 53.5 and 128.8 ± 52.8 ng ml^{-1} ($P = 0.06$, mean difference 54.4 ng ml^{-1}, 95% CI 24.8–84.0), respectively. Oral administration resulted in an earlier appearance of metabolite than after intravenous dosing (Figure 1b), and the ratio of the desmethyldiazepam to diazepam AUC was 10–20% greater for the oral route ($P < 0.005$ in both young and elderly groups, regardless of the

Table 1 Mean pharmacokinetic parameters of diazepam following single dose administration to young and elderly subjects

	CL_s (ml min^{-1} kg^{-1})	$t_{1/2}$ (h)	V_1 ($l\,kg^{-1}$)	V_{ss} ($l\,kg^{-1}$)	$AUCM_{iv}$ ($ng\,ml^{-1}\,h$)	CL_0 ($ml\,min^{-1}\,kg^{-1}$)	$AUCM_0$ ($ng\,ml^{-1}\,h$)	fu (%)	F (%)
Young ($n=7$)	0.29 ± 0.09	44.5 ± 16.5	0.24 ± 0.04	0.88 ± 0.30	166.23 ± 43.96	0.37 ± 0.22	2026.76 ± 680.07	2.49 ± 0.14	93.68 ± 36.69
Elderly ($n=6$)	0.26 ± 0.09	71.5 ± 27.6	0.23 ± 0.04	1.39 ± 0.32	118.23 ± 26.18	0.32 ± 0.18	1888.79 ± 929.60	2.76 ± 0.27	94.18 ± 38.39
P value	0.40	0.03	0.56	0.004	0.06	0.34	0.78	0.21	0.56

Data are mean ± standard deviation. CL_s, systemic clearance. $t_{1/2}$, terminal elimination half-life. V_1, initial distribution volume. V_{ss}, apparent volume of distribution at steady-state. $AUCM_{i.v.}$, area under the curve of desmethyldiazepam following intravenous administration. CL_0, apparent clearance determined following oral administration. $AUCM_0$, area under the curve of desmethyldiazepam following oral administration. *fu*, unbound fraction, *F*, apparent oral availability.

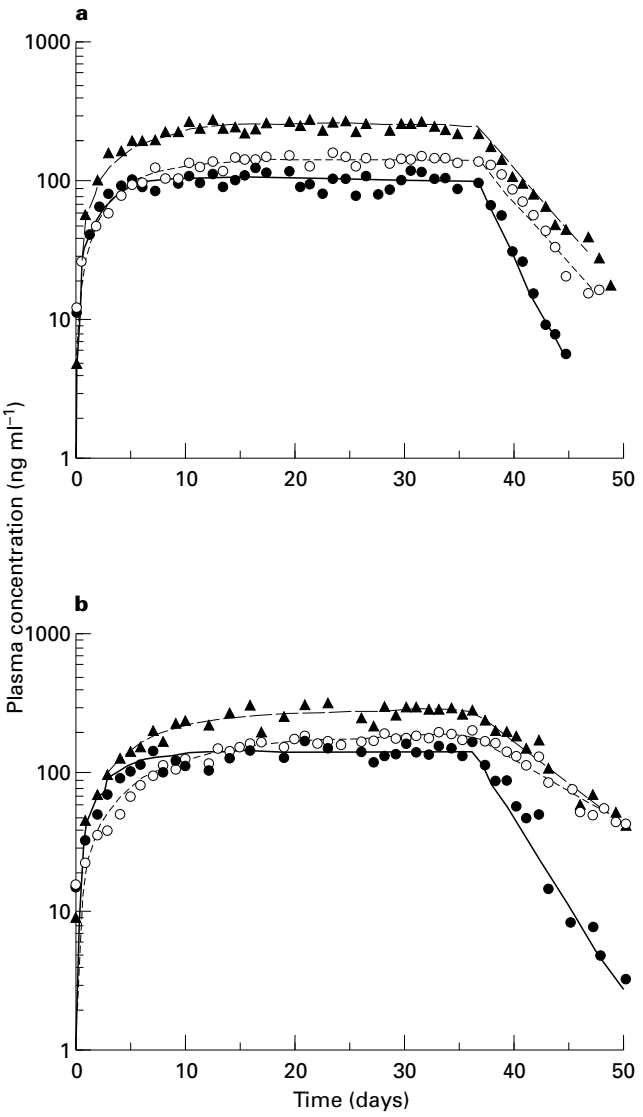

Figure 2 Time course of accumulation of mean plasma levels of diazepam (●), desmethyldiazepam (○) and 'benzodiazepine equivalents' (▲) in plasma of young (a) and elderly (b) subjects receiving 2 mg diazepam every 12 h for 6 weeks.

dosing protocol—see Table 2) in keeping with a modest first-pass effect. This ratio also tended to be lower in the elderly, although the differences were not statistically significant. The ratio of the dose-normalized desmethyldiazepam AUC after oral administration to that after intravenous administration was close to unity.

Radioreceptor assay results

The pharmacokinetics of 'benzodiazepine equivalents' as determined by the radioreceptor assay were different from those of diazepam itself. This would be expected, given the different dispositional characteristics of the compounds, predominantly diazepam and desmethyldiazepam, contributing to the estimation of the 'equivalent' concentration. For example, the rate of elimination of 'benzodiazepine equivalents' on discontinuing diazepam was intermediate between the rates of elimination of diazepam and desmethyldiazepam (0.0094 ± 0.0027 *vs*

Table 2 Ratios of the areas under the plasma concentration-time curves for desmethyldiazepam relative to diazepam following single- and multiple-dose diazepam administration to young and elderly subjects

	Single dose		*Multiple dose*	
	Oral	*Intravenous*	*Oral*	*Intravenous*
Young ($n=7$)	1.52 ± 1.06	1.01 ± 0.14[1]	1.43 ± 0.49	1.03 ± 0.12[1]
Elderly ($n=6$)	1.21 ± 0.70	0.74 ± 0.27[1,2]	1.22 ± 0.17	0.79 ± 0.30[1,2]

Data are mean $\pm$ s.d. [1]$P \leq 0.05$, oral *vs* intravenous. [2]$P \leq 0.10$, young *vs* elderly.

0.0173 ± 0.0061 and $0.0086 \pm 0.0037\ h^{-1}$, in younger subjects and 0.0070 ± 0.0040 *vs* 0.0117 ± 0.0051 and $0.0055 \pm 0.0036\ h^{-1}$, in the elderly). Also, the steady-state concentrations of 'benzodiazepine equivalents' approximated those of the sum of the two contributing compounds (243 ± 60 *vs* 225 ± 76 ng ml^{-1}, in the young; 288 ± 126 *vs* 331 ± 121 ng ml^{-1} in elderly; linear correlation coefficient $= 0.89$, $P < 0.001$). The apparent clearance of total bindable benzodiazepines after simultaneous intravenous and oral administration of diazepam were significantly reduced ($P < 0.05$) following multiple dosing in both young (0.16 ± 0.03 *vs* 0.23 ± 0.13 ml min^{-1} kg^{-1}) and elderly (0.13 ± 0.04 *vs* 0.29 ± 0.21 ml min^{-1} kg^{-1}) subjects. However, there was no effect of route of administration on any of the other parameters determined by the radioreceptor technique. Age was without effect on the disposition of 'benzodiazepine equivalents' (mean differences for the elimination rate constant $0.0024 \pm 0.0034\ h^{-1}$, 95% CI -0.0023–0.0071; for the steady-state 'equivalent' concentrations 45 ± 96 ng ml^{-1}, 95% CI -83–173; and for the apparent clearances, 0.03 ± 0.03 ml min^{-1} kg^{-1}, 95% CI -0.02–0.08).

An unexpected finding of the study was the presence of large, concordant secondary peaks in the diazepam and desmethyldiazepam plasma concentration-time profiles during the 12 h dosing interval following 6 weeks of chronic oral treatment with diazepam (Figure 3). These correlated in time with smaller peaks in the concentrations of simultaneously administered single-dose radiolabelled intravenous diazepam ($r = 0.813$, $P \leq 0.005$) and appeared to coincide with the first meal (4 h) after the overnight fast. The extent of diazepam fluctuation during the peaks was the same for the intravenous and oral dose ($29.2 \pm 20.6\%$). However, in the case of the intravenously administered diazepam they were more difficult to appreciate because the absolute concentrations were lower and because of the fact that they occurred while diazepam's disposition was still in its rapid distribution phase. Large post-prandial peaks also occurred in the concentrations of desmethyldiazepam ($31.7 \pm 15.1\%$) and, together with the diazepam peaks, produced correspondingly large peaks in the concentrations of 'benzodiazepine equivalents' ($32.8 \pm 22.2\%$). No concomitant changes were found in the concentration-time profiles of oxazepam or temazepam. Similar, but smaller, peaks were observed in many individuals after single dose oral diazepam administration. Like the comparisons of intravenous and oral diazepam peaking, the magnitude of these were the same as that observed during multiple-dosing.

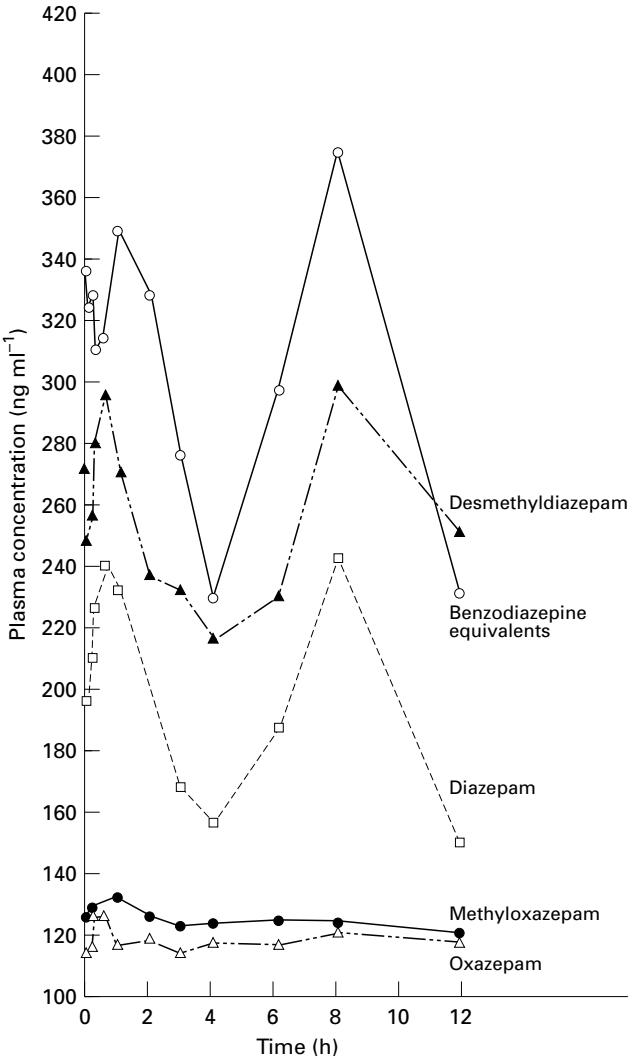

Figure 3 Steady-state plasma concentration-time profile of diazepam, desmethyldiazepam and 'benzodiazepine equivalents' showing the presence of large post-prandial peaks during the dosage interval. Data from an elderly subject.

Discussion

Age-related changes in the disposition of the benzodiazepines are of interest because of the wide usage of these drugs in the treatment of anxiety or insomnia in elderly patients. Moreover, epidemiological studies have revealed an increased propensity to adverse drug related events in the elderly [1–4], particularly for benzodiazepines possessing long elimination half-lives such as diazepam [22, 23]. Several studies have compared the pharmacokinetics of diazepam in young and elderly

subjects following acute, single-dose, administration and, in general, the findings of this study are in agreement with those of earlier investigations. An age-related decrease in the rate of elimination of diazepam has been consistently observed. Thus, it would be anticipated that the drug's steady-state plasma levels would be attained more slowly in elderly patients. The present experimental observations confirm that prediction.

The primary cause of the longer elimination half-life of diazepam appears to be an increase in the drug's volume of distribution in elderly subjects. Diazepam's plasma concentrations immediately following rapid intravenous injection were similar in the two age groups indicating that there were no differences in the initial volume of distribution of the drug (V_1). This finding is in contrast to an earlier report where V_1 was found to increase with age [5]. The discrepancy probably reflects the much earlier sampling protocol in the present study—the first sample being obtained 3 min after completion of the injection of diazepam rather than 15 min as in the earlier study, and a more rapid initial distribution of the drug in older subjects (Figure 1a). Accordingly, the age-related increase in distribution probably represents a difference in the more slowly equilibrating tissues, as measured by V_{ss}. Changes in the relative proportions of adipose and lean tissues with ageing may be responsible for the increase in the value of this pharmacokinetic parameter.

The elimination half-life of a drug is inversely proportional to its clearance rate from the systemic circulation. Thus, the prolongation in diazepam's half-life may also, in principle, reflect reduced clearance secondary to an age-related impairment in oxidative metabolism. Investigation of such a possibility presents a number of practical difficulties. For example, failure to collect plasma samples for a sufficient period of time to fully characterize the terminal elimination phase after a single dose, particularly for a drug like diazepam which possesses a longer elimination half-life in elderly individuals, may result in a higher estimate of clearance than would be determined during the dosage interval following the attainment of steady-state. Also, accumulation of diazepam or one of its metabolites could lead to inhibition of metabolism of the parent drug. Since there were no differences in our study in the oral clearance of diazepam following single-dose and multiple-dose administration in young or elderly age groups, it appears that neither of these factors were significant. Moreover, the similarity of the systemic clearance estimates after intravenous administration of a tracer dose of diazepam in the presence and absence of large accumulated concentrations of diazepam and desmethyldiazepam does not support the concept of auto-inhibition. The reason for the discrepancy between our findings and previous reports of inhibition of diazepam clearance following chronic dosing [11, 12] is not known. It may reflect the smaller doses of diazepam used in the present study, although the final plasma levels of desmethyldiazepam were comparable. Also, it should be noted that the extent of inhibition reported in these studies was modest and showed considerable interindividual variability [12].

A common problem in evaluating pharmacokinetic data is the large interindividual variability that occurs even in young healthy subjects. This may be further magnified by an increase in heterogeneity normally associated with ageing. Accordingly, results obtained from small study groups ($n = 6$ to 12) may reflect the subject selection process. Neither the systemic nor the oral clearance estimates in our study showed any statistically significant age-related differences. However, clearance values tended to be lower in the elderly and the steady-state plasma concentrations of diazepam were slightly higher in these individuals. The trend towards lower concentrations of desmethyldiazepam relative to diazepam in the elderly also supports a reduced clearance of diazepam due to impaired dealkylation. Given the fact that the magnitude of these differences was smaller than those previously reported (3% and 10% for multiple dosing and age compared with 30% and 40–50%, respectively) [7, 10, 11], it is possible that our study subjects were more homogeneous due to a more rigorous selection criteria (age and gender defined, non-smokers). Alternatively, our study may not have possessed sufficient power to demonstrate the differences statistically.

A *post hoc* analysis reveals that the likelihood of a Type II error in our study is quite large (0.89 for a dosing effect and 0.76 for an age effect) and the power to detect such differences is, correspondingly, low (0.11 for dosing, 0.24 for age). Indeed, to demonstrate a difference at a power of 0.80, assuming a variability of 30% and the same magnitude of change, would require a sample size greater than 250 for dosing and 100 for age. However, the problem is not with the study, itself, but rather in the appropriate framing of the questions asked in the study and the conclusions. Diazepam has a wide margin of safety and tolerance develops rapidly after long-term administration. A clinically important difference in clearance (one that would necessitate a change in diazepam dose) is probably around 30–50%. In this context, a more relevant question is whether or not our study has sufficient power to detect a clinically important difference of 30%. Given the *a priori* power analysis, this was clearly the case. Moreover, our radioreceptor assay results which take into account the greater concentrations of both diazepam and desmethyldiazepam at steady-state, showed no significant differences between young and elderly. Thus, it appears that ageing has only a limited, and clinically unimportant, effect on diazepam clearance.

The radioreceptor assay, which measures binding to rat cerebral cortex, provides an additional dimension to the assessment of pharmacokinetic and pharmacodynamic changes in *in vivo* systems and is particularly appropriate as applied to the characterization of diazepam disposition. Since binding at these receptors is similar to that reported for binding to benzodiazepine receptors in human brain [24], estimates of 'benzodiazepine equivalents' provide a reasonable measure of total pharmacological activity in the circulation at any given time and are likely to be a more meaningful reflection of the drug's clinical effect than measurements of diazepam, alone. However, they do introduce some

complexities in interpretation. For example, the steady-state plasma concentrations of 'benzodiazepine equivalents' were almost double those of diazepam, itself, and double those predicted by single-dose studies. At steady-state, the levels of desmethyldiazepam equalled or exceeded those of diazepam, itself. Accordingly, 'benzodiazepine equivalent' concentrations and the associated pharmacokinetic parameters represent an approximate 1:1 hybrid of the two component moieties. This is shown by the strong linear correlation between the 'equivalent' concentrations and the sum of diazepam and desmethyldiazepam concentrations and by the fact that the half-life of 'benzodiazepine equivalents' is intermediate between that of diazepam and its more slowly eliminated desmethyl metabolite. In contrast, after single-dose diazepam administration, desmethyldiazepam is present in only low concentrations relative to the parent drug and they accumulate slowly. Moreover, the receptor used in determining the concomitant 'equivalent' concentration has an intrinsically lower binding affinity for desmethyldiazepam. Thus, single-dose 'benzodiazepine equivalent' concentrations and pharmacokinetics primarily reflect the disposition of diazepam, alone. For this reason, there is an *apparent* accumulation of 'benzodiazepine equivalents' on multiple dosing that is due to the added contribution of desmethyldiazepam to the biological (radioreceptor) assay. Of course, there is no true accumulation above that which would be expected, given the accumulation of metabolite. However, the point is that the body experiences the same exposure as the receptor experiences, and that suggests an accumulation. These findings emphasize the need to consider the overall bioactivity profile when active metabolites are formed and accumulate.

An unexpected finding was the presence of large postprandial diazepam and desmethyldiazepam fluctuations during the multiple-dose phase of the study. Although a similar phenomenon has been reported following single-dose diazepam administration [25–29], the magnitude of these peaks appeared to be much greater than that formerly described. The mechanism(s) responsible for the peaks is unclear. However, given that both diazepam and its desmethyl metabolite have long elimination half-lives, it is unlikely that such rapid changes in plasma concentrations involve changes in clearance. Enterohepatic recycling is also unlikely since only a small amount of diazepam is excreted in bile [30–32]. Fluctuations in diazepam plasma levels have been observed following meals and major physiological stress such as parturition [33], and it has been suggested that changes in diazepam's plasma protein binding result in redistribution of drug from the tissues to plasma [34–36]. Unfortunately, binding was not measured during the period of actual peaking in the present study. Nevertheless, it is clear that such fluctuations, regardless of their cause, contribute greatly to the observed intersubject variability in diazepam's pharmacokinetics.

In summary, the disposition of diazepam is altered in elderly subjects. This is manifested primarily as a prolongation in the elimination half-life of the drug and is due to corresponding increases in steady-state volume of distribution. Clearances may also be slightly reduced, although these differences are small compared with interindividual differences in drug elimination and are not likely to be clinically relevant. On the other hand, accumulation of diazepam and desmethyldiazepam are extensive during chronic dosing. There are no differences in diazepam kinetics between single-dose and multiple-dose administrations and no differences between young and elderly subjects. However, consideration of the total benzodiazepine levels (drug and metabolites), particularly with inclusion of desmethyldiazepam, suggests that accumulation results in large increases in bioactive drug in the circulation of patients following chronic oral dosing.

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